AD-A197 391

PORT DOCUMENTATION PAGE

1 (U)	1b. RESTRICTIVE MARKINGS NA
2a. SECURITY CLASSIFICATION AUTHORITY NA	3 DISTRIBUTION / AVAILABILITY OF REPORT Distribution unlimited.
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE NA	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) Massachusetts Institute of Technology.	5. MONITORING ORGANIZATION REPORT NUMBER(S) NA
6a. NAME OF PERFORMING ORGANIZATION: Massachusetts Inst. of Technology. / 6b. OFFICE SYMBOL (If applicable): NA	7a. NAME OF MONITORING ORGANIZATION: Office of Naval Research
6c. ADDRESS (City, State, and ZIP Code): Harvard-MIT Division of Health, Sciences and Technology, Massachusetts Institute of Technology, 77 Massachusetts Ave., Cambridge, Mass. 02139	7b. ADDRESS (City, State, and ZIP Code): 800 N. Quincy Street Arlington, VA. 22217-5000
8a. NAME OF FUNDING / SPONSORING ORGANIZATION: Office of Naval Research / 8b. OFFICE SYMBOL (If applicable): ONR	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: N00014-87-K-0479.
8c. ADDRESS (City, State, and ZIP Code): 800 N. Quincy Street Arlington, VA. 22217-5000	10 SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO
61153N	RR04108	441K709	

11. TITLE (Include Security Classification)

(U) Electroporation: Theory of Basic Mechanisms

12. PERSONAL AUTHOR(S)

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13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual Project	FROM 06-01-87 TO 05-31-88	88/6/30	3

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	electroporation, bilayer membranes, cell membranes, membrane channels, bioelectrochemistry.

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The dramatic and apparently universal phenomenon of electroporation has implications for basic understanding of cell membranes, and is also likely to lead to a number of new applications. To date, however, a quantitative understanding of how electroporation occurs has been lacking. We report significant progress towards providing descriptions of mechanisms which can quantitatively account for some key features of electroporation, and also on related issues concerning the magnitude of applied electrical fields which should be detected by cells.

20 DISTRIBUTION / AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	U

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
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DD FORM 1473, 84 MAR — 83 APR edition may be used until exhausted. All other editions are obsolete. SECURITY CLASSIFICATION OF THIS PAGE

Date: 30 June 1988

PROGRESS REPORT ON CONTRACT N00014-87-K-0479

PRINCIPAL INVESTIGATOR: James C. Weaver

CONTRACT TITLE: Electroporation: Theory of Basic Mechanisms

DATE: 30 June 1988

RESEARCH OBJECTIVE: The objective of our investigation is to develop a theory of the mechanism of electroporation, ~~with the more specific objective of~~ providing a quantitative description of key features of this dramatic phenomenon. Electroporation: (1) is now believed to be a universal cell membrane phenomenon, involving both the lipid bilayer and membrane macromolecules, (2) provides a general method for introducing molecules into cells, or releasing molecules from cells, with potentially major applications in science and technology, and yet (3) is presently poorly understood. For example, no previous theory (other than our early work) actually describes electrical behavior during electroporation, membrane recovery, or the amount of molecular transport. With this in mind, our specific goals are:

(1) Extension of our first, successful theory of reversible electrical breakdown to one with more solid foundations, i.e. elimination of the approximate "switch on" criteria of pores. Such an extension should be based on a "continuous conduction" description which includes both Born energy effects as well as the obvious geometric size constraints of a pore.

(2) Development of a theory which describes quantitatively the transmembrane potential, U(t), during irreversible rupture, such that a unified theory of both REB and rupture is provided by one model. Such a theory should yield predictions of U(t) which can be compared directly with experiments, a basic requirement of which has not yet been achieved by other theories of electroporation.

(3) Extension of our first, successful theory of the reversible electrical breakdown of electroporation to include metastable pores associated with a pore-membrane macromolecule interaction. Membrane channel proteins are prime candidates for such interactions, and may provide nucleation sites for the long lifetime pores which are believed to occur. Such metastable pores should have significant lifetimes (seconds to minutes) which are temperature dependent.

(4) Development of a more complex theory which also predicts the amount of transmembrane transport of molecules. Here the involvement of membrane proteins, particularly channel forming proteins, is believed to be important. Such a theory should include hindered diffusion as a primary mechanism, particularly for long times involving persistent metastable pores, but should also include mechanisms such as electrophoresis and electroosmosis which can operate during the time the transmembrane potential is non-zero. The general goal is to predict the number of molecules which move across a cell membrane.

Throughout we will seek quantitative comparisons between the predictions of theory and the results of experiment.

Keywords: Membranes biology, Biochemistry, Electric Fields, Membrane, Cell, Protein, Electrophoresis, Electroosmosis, Bioelectrics

PROGRESS

We have made significant progress towards several of these objectives, with two scientific manuscripts in preparation, and additional progress towards another major objective. More specifically,

(A) We have extended our initial theory of electroporation to provide an improved theory of reversible electrical breakdown (publication 6.). This version of a transient pore theory eliminates the use of sharp conduction criteria for pores, and instead uses a more realistic continuous conduction in which an estimate of the Born energy is used with a Boltzmann factor to describe the reduced conductivity of a pore in a low (compared to water) dielectric constant membrane. This version also eliminates the use of an assumption

concerning the number of pores present in a membrane at equilibrium, and instead utilizes creation and destruction rates for pores. This version further assumes a realistic minimum pore size, based on molecular sizes.

(B) We have obtained a unified theory which provides a quantitative description of U(t) during REB and also of U(t) during rupture (publication 7.), with both descriptions in good agreement with experiments of others. This unified theory utilizes the description of reversible electrical breakdown described in (A), and also a description of the contribution of one "run away" pore (leading to rupture) to the membrane resistance, and thereby to dominating the transmembrane potential, U(t). This is such first unified and quantitative description of both of these dramatic electroporation phenomena.

(C) We have also made progress towards combining these improved theories of the very early electrical events (reversible electrical breakdown and electrical rupture) with a model for pore-membrane protein interactions. We expect that this on-going work will lead to a reasonable description of cell membrane electroporation, wherein first dramatic electrical events occur, followed by two phases of membrane recovery (fast, complete resealing of most pores, and slow, metastable pore-protein complexes which recover through a thermally activated process). We further expect that mass transport through both the fast recovering pores and slowly recovering pores will be significant, with a combination of hindered diffusion and electrokinetic transport being significant for the fast recoverying pores, and primarily hindered diffusion being important through the slowly recovering pore-protein complexes.

PUBLICATIONS AND REPORTS

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4. A. Barnett, K. T. Powell and J. C. Weaver (1988) "Electroporation of Artificial Bilayer Membranes: A Quantitative Theory" Abstract and poster paper, 10th Ann. Meeting, Bioelectromagnetics Society, June 19 - 23, Stamford, CT.

5. J. C. Weaver and K. T. Powell (1988) "Theory of Electroporation" in Electroporation and Electrofusion in Cell Biology, E. Neumann, A. Sowers and C. Jordan (Eds.), Plenum, New York (in press).

6. A. S. Barnett and J. C. Weaver "Electroporation: A Continuous Conduction Theory of Reversible Electrical Breakdown" (in preparation).

7. A. S. Barnett and J. C. Weaver "Electroporation: A Unified, Quantitative Theory of Reversible Electrical Breakdown and Rupture" (in preparation).